ASM Position Paper on BWC Verification
Draft November 1, 1996

The American Society for Microbiology (ASM), the largest single life science organization in the world representing 32,000 microbiologists from the United States and over 11,000 microbiologists from other countries around the world, has a long history of involvement in debates about biological weapons. Members of the ASM bring a critical base of scientific and technical knowledge to issues related to biological weapons, human health, the environment and global security. The ASM has served an advisory role to governments on issues related to biological weapons. In 1994, the ASM's Public and Scientific Affairs Board established a Task Force of expert scientists to assist in developing scientifically sound approaches to biological arms control. This Task Force has considered the scientific and technical measures that could be included in verification regimes aimed at strengthening the Biological Weapons Convention (BWC).

The ASM has a long standing position that biological weapons should not be developed and that steps should be taken at the international level to prevent the development and use of biological weapons for warfare and terrorist activities. As such, the ASM strongly endorses efforts to develop verification regimes with sound scientific underpinnings which will act as effective deterrents to the development and use of biological weapons and which will increase global security and human well-being. The ASM Task Force believes that the technical means exist for the development of verification regimes under the BWC that would enhance global security and would not deter legitimate scientific research and development and jeopardize confidential business information or result in the loss of proprietary microorganisms or biodiversity resources. The Task Force believes that verification regime should include provision for notification and investigation of all unusual disease outbreaks, confidence building of compliance with the provisions of the BWC through declarations and routine visits, and challenge inspections that include sampling and analysis when there is sufficient evidence to warrant such inspections.

Surveillance of Disease Outbreaks

Policy Considerations

Surveillance of disease outbreaks serves the dual purpose of enhancing global security by providing a warning network for the detection and control of epidemics and by establishing an epidemiological data base against which unusual disease outbreaks that could be associated with biological weapons development or use can be assessed. Various agencies such as the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) already are engaged in investigating disease outbreaks and have established epidemiological data bases and investigative methodologies. The BWC could enhance the investigative and epidemiological capabilities of an agency such as WHO so as to ensure that any disease outbreak associated with biological weapons development would be detected and investigated and that the global epidemiological data base or disease occurrences and geographic distributions of pathogens were adequate for monitoring compliance with the BWC. Enhancing epidemiological data bases would enhance the capability of distinguishing biological weapons from naturally occurring pathogens.

Scientific Underpinnings

Most unusual disease outbreaks will be naturally emerging diseases but could be the result of accidental or intentional release of biological weapons agents. Differentiating the source of disease outbreaks often is difficult. Adequate epidemiological data bases that provide background data are important for making determinations about the source of a disease outbreak.

Procedures used by the CDC and WHO and personnel from those organizations, for example, those used during the 1995 outbreak of Ebola in Zaire, are well suited for such epidemiological investigations. During such investigations cultures and specimens containing viable microorganisms are collected. These are shipped with appropriate containment for transport of pathogens to high containment laboratory facilities where identifications can be made. These procedures should be followed in investigating all disease outbreaks.

Using the personnel and facilities of the World Health Organization as well as national diagnostic

laboratories is preferable to developing an independent infrastructure for biological weapons verification. However, it should be emphasized that in order to detect a disease outbreak due to natural causes or a biological weapon, it is absolutely essential to have both a national and global infectious disease surveillance system. Currently, we have neither. To protect the world from biological weapons, the establishment of effective disease surveillance programs must be given the highest priority.

The greatest extent of experience in searching for biological agents that cause disease is in the medical and agricultural fields where epidemiological investigations are routinely conducted. Both the success and problems with various approaches to sampling and analysis can be seen in epidemiological investigations of unusual disease outbreaks. While past biological weapons development programs point to the most likely microorganisms and toxins for which to search and human intelligence gathering does point toward when and where to inspect, it is these epidemiological investigations of disease outbreaks that give us the scientific intelligence of how to sample and analyze those samples for the detection of biological weapons. Most unusual disease outbreaks are due to naturally emerging infections, sometimes with previously unrecognized pathogens.

Locating the source of a disease outbreak often is difficult. There frequently is a lack of adequate epidemiological data for determining the source of a disease outbreak. Efforts by the CDC and the WHO and other teams of medical investigators, for example, have yet to determine the source of the 1995 outbreak of Ebola hemorrhagic fever in Zaire. While there have been claims that the Ebola virus is the result of Soviet biological weapons development, it is far more likely that outbreaks of Ebola are natural occurrences that have been fostered by human development in previously remote regions that bring humans in contact with previously unrecognized pathogens. Finding the reservoir of the Ebola virus, most likely within primate animals in the jungle, may take years of exhaustive and expensive epidemiological investigation.

irresponsible

In similar fashion, when the first outbreak of Legionnaires disease was recognized in 1976 during

a conference of the American Legion in Philadelphia, there were early claims that the disease outbreak was the result of terrorist activities. It took years of research costing millions of dollars to establish that the disease was caused by a naturally occurring bacterium *Legionella pneumophila* which is widely distributed in water. Now that we know the causative organism, its reservoir, and its mode of transmission, epidemiologists are able to rapidly diagnose outbreaks of Legionnaires disease and to determine the most likely source underlying the infection so that further spread of the disease can be halted.

Technical Considerations

A. Notification of unusual outbreaks of disease due to infections should be sent immediately to a designated responsible agency. The notification should include the following information:

1. Name and address of responsible office and responsible health official (including telephone and fax numbers):
2. Location and area (km2) affected
3. Date of recognition; duration (weeks)
4. Numbers of cases
   (i) Morbidity
   (ii) Mortality
5. Main clinical signs
6. Cause (*microorganism or toxin*)
   (I) Suspected
   (ii) Laboratory proven
   (iii) WHO risk group (III, IV or no)
7. Why is the disease outbreak considered unusual?
8. Has the disease occurred before? If so what was the incidence of morbidity and mortality

B. Investigation of Unusual Disease Outbreaks

1. Investigations should be carried out by trained public health scientists such as

1. Investigations should be carried out by trained public health scientists such as those of WHO, CDC, and other established public health agencies
2. Normal epidemiological investigation methodologies should be employed during such investigations. These may include sampling and analysis of viable cultures as well as serological and genetic based analytical procedures.
3. Data from such investigations should be entered into a global disease surveillance network.

Dr. Morse should expand this section.

Confidence Building Measures: Declarations and Routine Visits

Policy Considerations

Declarations and routine visits can help build confidence that nations are complying with the prohibition of developing biological weapons by increasing the transparency of activities at various facilities that deal with pathogens and that might have the capacity for production of biological weapons and systems for the dissemination of biological weapons. Declarations and routine visits may also act as an effective deterrent to the development of biological weapons. Declarations and routine visits are likely to detect technical violations of a BWC verification protocol--for example omissions of items that should be declared but are unlikely to detect any actual biological weapons or evidence for development of such weapons. Routine visits could disrupt normal operations at a facility and could result in loss of confidential business information and proprietary microorganisms unless they are properly managed and access to the facility is carefully controlled. For cost effectiveness and to minimize the risk of loss of confidential business information it is important to keep the number of institutions and companies that must declare to a justifiable level. It is also important to limit sampling during routine visits to those that can establish that the activities of the facility are consistent with those that have been declared.

Scientific Underpinnings

Given that verification of the Biological Weapons Convention centers on prohibiting the

of possessing pathogens that are considered potential biological weapons and the production capacity to cultivate those organisms within a facility should be the criteria for defining who should be required to file declarations. Verification of the Biological Weapons Convention differs significantly from verification of the Chemical Weapons Convention in that biological weapons are based upon microorganisms that are capable of reproduction or replication and which naturally occur and cause disease. Microorganisms that might be employed as biological weapons are widely distributed in nature and are routinely cultured (grown) in clinical diagnostic laboratories. Some pathogenic microorganisms are also cultured for the production of vaccines to prevent disease. Many are grown in academic, government, and industrial laboratories for scientific studies on how they cause disease and how they can be controlled. Pathogens are routinely cultured in clinical laboratories for the diagnosis of infectious diseases.

Development of lists of organisms of concern as possible biological weapons agents and facilities/equipment that might be used for producing or disseminating biological weapons would help delineate declaration procedures. Such lists would apply only to declarations and not to other components of the BWC verification regime. Assuming that a list of organisms that would require declarations is developed, it should be regularly reviewed and updated and should include provision for genetically modified organisms containing virulent genes from listed pathogens of concern. Within a single institution or company, the requirement to file a declaration should be based on the presence of a specific pathogen on the selected list of potential biological agents coupled with the capacity to grow large enough volumes of that organism to be used as a biological weapon. This should exempt all clinical laboratories involved exclusively in the diagnosis of disease from the declaration process. Relatively few facilities handling pathogens have high volume production capacities. Careful consideration will have to be given as to whether the capacity of an individual facility or some combined capability of multiple facilities is considered in deciding what facilities must file declarations. Routine visits could be conducted to verify the accuracy of declarations. Such visits would increase transparency so as to help deter the development of biological weapons. Routine visits would be aimed at developing confidence that a facility was carrying out the activities that it declared and was carrying activities consistent with

those not precluded by the BWC. Routine visits are unlikely to detect biological weapons as such detection almost always would require very invasive sampling and analysis procedures that would be disruptive to the operation of any facility and could not be justified as part of a routine confidence building activity.

A visit that included visual observation of the facility would indicate to trained observers that the equipment was consistent with legitimate activities. Viewing of records would permit a gross audit of input and output that would build confidence of compliance with the BWC. Sampling of the end products would permit confirmation that the facility was producing products consistent with its declared activities. Sampling and analysis of end products would not disrupt ongoing activities of the facility and would pose no risk of loss of proprietary microorganisms or confidential business information.

Technical Considerations

A. Who should complete declarations for biological weapons verification?
   1. All signatories to the BWC
   2. All government supported facilities (including government contractors) working on defense against biological weapons.
   3. All installations with fermentation capacities above 10 liters per day.
   4. All installations working at the BL4 or BL3-LS levels.
   5. All containment facilities for work on aerosol exposure.
   6. All facilities working with class III or class ~V pathogens.
   7. All facilities working on human or animal vaccines.

B. When and to whom should declarations be submitted?
   1. Declaration should be completed annually.
   2. Each member government should compile all declarations from facilities of that nation and submit a single unified declaration to the secretariat by April 30 of each year.

C. What Information Should be Contained in an Overall Declaration

1. Date of Declaration
2. Name of State Party to the Convention:
3. Name and address of responsible office and responsible official (including telephone and fax numbers):
4. For each of the following, indicate if there is something to declare and if so, describe briefly; indicate the location (specific address), responsible official (include address, phone number, and fax number). For each facility identified a separate individual declaration should be completed.
   - (i) BL4 or BL3-LS containment facilities
   - (ii) Containment facilities equipped for aerosol studies
   - (iii) Facilities working with class III or class IV pathogens
   - (iv) Government supported facilities (including government contractors) working on defense against biological weapons
   - (v) Installations with fermentation capacities above 10 liters per day
   - (vi) Facilities working on human or animal vaccines

D. What Information Should be Included in Declarations by National Defense Program Facilities

1. Name and address of responsible office and responsible official at facility location (including telephone and fax numbers):
2. Source of funding.
3. Area occupied by facility (m2 area of site).
4. Floor area occupied by buildings at facility (m2).
5. Annual cost of operating facility at site (US$)
6. Number of scientific and technical personnel (indicate numbers of full and part-time, both in-house and working under contractual arrangements).
7. Activities (describe)
8. Names of all microorganisms or toxins used.
9. Publications from work associated with the program, indicating affiliations of each author at the time of publication.

10. Visits to facility by scientists from other countries.
11. Visits to other facilities by personnel from this National Defense Program.
12. Titles, dates and locations of international conferences, seminars, or symposia on the subjects contained in the national biological defense research and development program, which staff have attended during the year preceding the date of this declaration.
13. National legislation and regulation relating to biological weapons (supply copies of legislation considered to fill the obligation of Article IV of the BWC).
14. Past programs intended to develop or produce biological weapons.
15. Past defensive biological weapons programs

E. What Information Should be Included in Declarations by Vaccine Production Facilities

1. Name and address of responsible office and responsible individual at facility location (including telephone and fax numbers):
2. Vaccines produced
3. Annual production in doses (give for each vaccine produced)
4. Number of scientific and technical personnel (indicate numbers of full and part-time, both in-house and working under contractual arrangements)
5. Production capacity (liters)
6. Publications from work associated with the facility, indicating affiliations of each author at the time of publication.

F. What Information Should be Included in Declaration by facilities with BL4, BL3-LS Containment or Aerosol Chambers

1. Name and address of responsible office and responsible official at facility location (including telephone and fax numbers):
2. Number of containment units.
3. Size of units (m2 of floor area inside containment unit).
4. Function (research, diagnosis, production: development and testing of protective equipment).
5. Name of microorganisms or toxins used during the preceding year.

6. Number of aerosol chambers, if any.
7. Purpose of aerosol chambers.
8. Publications from work associated with the facilities, indicating affiliations of each author at the time of publication.

G. What information should be included in declarations by installations with fermentation capacities above 10 liters per day.

1. Name and address of responsible office and responsible individual at facility location (including telephone and fax numbers):
2. Activities (describe purpose of work)
3. Organisms grown in facility.
4. Number of fermentation units.
5. Production capacity (liters)
6. Media (Type used and amounts consumed)
7. Publications from work associated with the facility, indicating affiliations of each author at the time of publication.

H. What Information Should be Included in declarations by Facilities working with class III or class IV pathogens

1. Name and address of responsible office and responsible individual at facility location (including telephone and fax numbers):
2. Name of microorganisms or toxins used during the preceding year.
3. Activities (describe purpose of work with each pathogen)
4. Funding (amount and source)
5. Media (Type used and amounts consumed)
6. Sources of cultures
7. Sites where cultures have been sent
8. Publications from work associated with the facility, indicating affiliations of each author at the time of publication.
9. Visits to facility by scientists from other countries.
10. Visits to other facilities by personnel.

10. Visits to other facilities by personnel.

Dr. Zilinskas write a section on routine visits and what activities would be appropriate during such visits, e.g. audits of records to establish an input/output balance, visual inspection of equipment, sampling and analysis of end products--this could include discussion of how to protect CBI during such visits--perhaps endorse the PHARMA approach of managed access for this section.

Challenge Inspections: Sampling and Analysis

Policy Consideration

Detection capability is an essential investigative tool in cases of alleged or suspected uses of biological weapons. Global and national security requirements will mandate that when there is adequate evidence that biological weapons are being developed or have been used inspections will be needed to refute or confirm that evidence. Such inspections will require sampling and detection procedures that are accurate so that the results are clear and irrefutable.

There should be a balance between the need to detect violations of the BWC (benefit of detecting actual development or use of biological weapons) and the costs of verification activities. Inspections that ensure detection of any illicit biological agents are clearly beneficial for global multinational security. Inspections that fail to detect biological weapons properly, either because of false negatives or false positives would be very costly. Also among the costs of carrying out inspections, it is necessary to include consideration of the need to protect against the loss of natural biodiversity resources of a nation, the loss of proprietary assets of industry, and the loss of intellectual property of researchers. While a necessary component of verification procedures for the BWC, challenge inspections with invasive sampling procedures should only be employed when there is adequate evidence and should not heighten the risks to human health through the spread of disease-causing microorganisms. Sampling also should be conducted in ways that minimize the risk of loss of proprietary microorganisms and confidential business information, national security information, and biodiversity resources.

Unlike the CWC, the Biological Weapons Convention focuses on the detection of microorganisms and their toxins. Removal of a viable microorganism is tantamount to removing an entire factory and its operating instructions Many microorganisms are studied in research laboratories and many are used in industrial processes. Some of these organisms are a valuable national asset; some are proprietary to industrial concerns and of significant economic value. Preventing the loss of such valuable microorganisms is important and there must be a balance between protecting the natural biodiversity resources of a nation, the proprietary assets of industry, the intellectual property of researchers on the one side and on the other side protecting the world (multinational concerns) against the possible development and use of biological weapons. Methods should combine detection (verification) with protection of proprietary microorganisms and the incentive for scientific research and development. This approach can be accomplished using on site analysis of nonviable microorganisms with existing methodological approaches, employing both genetic and immunological procedures.

Scientific Underpinnings

Sampling and analysis procedures employed in inspections to verify compliance with the BWC must be safe and accurate. They must ensure the safety of the public and the inspectors as well as provide assurance to the general public that any biological weapons development can be adequately detected so as to provide a measure of public safety. To provide the necessary accuracy and to understand the limitations of sampling and analysis procedures, it will be necessary to develop and to fully test these procedures. Only in that manner can reliable (verifiable) tests be used in which confidence in the scientific accuracy of test results can be placed. The development and validation of test procedures will establish a de facto list of organisms that can be detected during an inspection. As long as no official list of prohibited organisms is established in a BWC verification protocol, the de facto list can be regularly modified as new tests are developed and validated.

Regardless of the sensitivity and specificity of the sampling and analysis procedures, there is a high risk that inspections will fail to detect biological agents, even when they are being developed,

as evidenced by the UNSCOM inspections of facilities in Iraq after the Gulf War, or to properly associate pathogens and disease resulting from biological weapons activities, as evidenced by the initial investigations of the release at Svedlosk in the former Soviet Union. There is also a high risk that pathogens that could be used as biological agents will be detected even though they are not the result of biological weapons-related activities. Clearly, having scientific intelligence about pathogens and their normal routes of dissemination allows medical investigations to determine to the cause of disease outbreak. Knowing the identity of a specific pathogen and the natural transmission route of a disease makes intelligent sampling and analysis possible. Such epidemiological knowledge underpins the ability to determine that an unusual disease outbreak is from natural sources or may be due to illicit activities associated with biological weapons. Methods that provide the greatest confidence for the detection of biological weapons and compliance with the BWC are likely to be the most intrusive and to pose the greatest risks for loss of proprietary organisms and industrial proprietary information. Making inspectors and the administrative bureaucracy involved in such inspection accountable for protecting confidential business information lowers but does not totally eliminate potential losses to national industries. Lowering the degree of intrusiveness lessens the costs to industry but also lowers the confidence in the inspection process and results. Superficial inspections with limited sampling and analysis will not provide great confidence that failure to detect suspected biological agents supports the conclusion that a nation is not conducting biological weapons development activities. Human intelligence data is needed to know where to conduct inspections (where to sample) and the biological agents at which the inspection is aimed at detecting (what analyses to conduct). Inspections at best can confirm allegations of biological weapons development or use. As such they are most effective as challenge inspections where substantive intelligence data points to violations of the BWC.

Because of major advances in technology, it should be possible to provide the transparency necessary to ensure compliance with the Biological Weapons Convention without jeopardizing the proprietary nature of some microorganisms by using on-site analysis of nonviable microorganisms. There should be no need to remove live or viable microorganisms in order to accomplish the task

of identifying microorganisms at a site because of the technology that is now available. Precluding off site transport of viable cultures also provides a measure of safety in the event that a sample actually containing a viable biological weapon agent could leak during transport or mishandling during analysis, causing a significant disease outbreak and fatalities.

Technical Considerations

In carrying out sampling for potential detection of biological agents, great care must be taken to avoid exposure to samples that may contain deadly pathogens. It must be assumed that samples may contain biological agents capable of causing serious morbidity or mortality. Therefore, sampling and analyses must be conducted in BL-4 equivalent containment. Very high costs will be incurred in ensuring that inspectors and others are not inadvertently exposed to deadly pathogens. Minimizing the risks associated with sampling necessitates using field suits and laboratories that avoid exposure of personnel to potential biological agents that have been developed as weapons. The cost of deploying such facilities is great as shown by the investigations of the Ebola outbreak in Zaire. Shipment of samples containing viable organisms and analyses of such samples also must be conducted with maximal containment. Analyses that can be conducted at the site and those which employ samples with killed microorganisms greatly lower the risks of exposure of inspectors and the general public to any biological agents in the samples.

In carrying out sampling for investigating possible development or uses of biological weapons it is necessary to take into consideration the environment where the evidence (pathogens or toxins) may be found. Very different procedures are employed when examining air, water, soil, cultures, tissues, and other media that may contain pathogens or toxins. Specific sampling procedures aim to provide sufficient concentrations of toxins, pathogens, or biochemicals used for detection (identification) of biological weapons. Quantities of samples adequate for detecting biological weapons will depend upon the sensitivity of the specific analytical procedure to be used, the likely concentrations of pathogens or toxins in the samples, and the efficiency of recovery.

The costs and benefits of sampling procedures must be coupled with the analytical procedures that

will be employed. As a rule the greater the number of samples collected and analyzed the greater the reliability of the results. However, the cost of collecting and analyzing a greater number of samples is higher than the costs of sampling and analyzing a lower number of samples. Analyzing more samples also does not increase the benefits if the samples are not of adequate quantity and quality to support the analytical procedures used for detecting pathogens and toxins.

Generally air has very low concentrations of microorganisms and therefore very high quantities of air must be filtered in order to collect sufficient concentrations of material for analysis. Air sampling generally only will prove useful during a time period when there is an actual spread of biological weapons, for example, during an accidental release or actual use of such agents. Microorganisms can be recovered from air by filtration for later analysis. Sampling of water from natural sources, such as potable water supplies, is likely to prove useful for detection of biological weapons only during actual uses or accidental releases. Even in cases where biological weapons were actually used the concentrations in natural waters would be very low due to dilution. Given the likely dilute concentrations of pathogens in such samples, it would be necessary to collect samples ranging from a few hundred ml to hundreds of gallons would be needed for analysis. This would make detection very difficult. A greater likelihood of finding pathogens or toxins intended for dissemination of biological weapons would be in storage vessels containing agents or within actual weapons. Here high concentrations of pathogens or toxins would be expected and hence low volumes, a few milliliters, would provide a sufficient sample for analysis. This points to the greatest benefits in collecting samples from lots of vessels within suspected production and storage facilities. This also points to the greatest concern by industry, that is, the collection of many samples that will lead to potential loss of proprietary organisms from reactors and vessels used in production processes.

Lowered risks of significant industrial costs and greater benefits are likely to occur when samples are obtained from humans or other animals that are suspected of having been exposed to biological weapons-related agents. The easiest samples to analyze, in terms of quantities and comparative data, will come from diseased humans, animals, or plants. Here amplification of a

pathogen would have occurred during the course of infection and disease so that evidence of pathogens would be a concentrated in body fluids or tissues that could be readily sampled. In some cases samples would be recovered from ill or recovering individuals, but in others the samples would be obtained during autopsy. Epidemiologists have extensive experience in the recovery of pathogens from tissue or body fluid samples as this is the approach most often used for definitive diagnosis of infectious diseases.

Four analytical approaches can currently be employed for the detection of potential biological weapons. These are (1) culture of viable microorganisms followed by morphological, physiological, and biochemical tests to identify the microbial species sometimes augmented with molecular or serological tests to determine the specific strain; (2) genetic analysis based upon the molecular biology of the microorganism for diagnosis; (3) immunological (serological) analysis based upon the antigenic properties of the microorganisms or toxin that permits its specific identification; and (4) direct chemical analysis based upon the ability to detect unique biochemicals associated with specific pathogens and toxins. Each of these methodological approaches has its specific sample requirements, strengths, limitations and costs (Table 1). As a result, individual cost benefit analyses are required for each analytical approach.

Table 1. Strengths, Limitations, and Costs of Various Analytical Detection Approaches

Method	Strengths	Limitations
Culture	Standard procedure for epidemiological investigations; adequate data base for comparisons; provides material for additional confirmatory analyses	Requires appropriate culture medium and conditions; requires viable microorganisms; not all microorganisms can be cultured; doesn't discriminate from natural pathogens; requires days to weeks of culture; elevated risk to workers; elevated risk of loss of proprietary microorganisms; not applicable for toxin detection
Genetic (PCR/gene probes)	High degree of specificity; high degree of sensitivity; rapid; doesn't require viable microorganisms; doesn't require high level containment for safety; rapid (minutes to hours); specific; preserved samples can be analyzed; low risk to workers	Requires knowledge of appropriate target sequence; interference by various agents in soil and tissues; very target specific; requires purified target nucleic acids; doesn't indicate viability of microorganisms; not applicable for toxin detection; risk of loss of proprietary genes
Immunologic (serology)	High degree of specificity; high degree of sensitivity; rapid; doesn't require viable microorganisms; doesn't require high level containment for safety; rapid (minutes to hours); specific; applicable for toxin detection; low risk to workers; negligible risk of loss of proprietary material; low concentrations of target molecules if amplification procedures such as ELISA are employed; no viable microorganisms needed	Requires knowledge of appropriate target; interference by various agents in soil and tissues; highly target specific
Chemical	Rapid detection method that may have adequate specificity; may be coupled to computer chips for rapid electronic detection; does not require viable microbes; negligible risk of loss of proprietary material; ; specific; preserved samples can be analyzed; low risk to workers; relatively low concentrations of pathogens may be detected.	Relatively new approach to identifying microorganisms so that pitfalls have not been completely elucidated;

Culturing viable microorganisms is the classic approach that is used world-wide for the identification of pathogenic microorganisms. Such analyses are the mainstay of epidemiological investigations by organizations like the CDC and the WHO. Hence, there is a great deal of comparative data available and experience in identifying viable cultures of microorganisms.

When viable culture approaches are employed it is essential that the sampling and shipping procedures maintain the viability of the microorganisms in the sample. This requires knowledge of the conditions necessary for maintaining the viability of the specific pathogen(s) in the sample. Knowledge of the specific suspected pathogen is critical from the time of sample collection. Elevated temperature, exposure to air, and a variety of other factors may kill specific pathogens in a sample and appropriate steps, such as maintenance in the absence of air, must frequently be employed for the recovery of specific pathogens.

Knowledge of the specific suspected pathogen also is necessary for selecting the appropriate culture medium. Each pathogen has specific culture requirements designed to meet its physiological needs. Many pathogens are fastidious, meaning that they require highly specific nutrients and physical conditions for reproduction. Often they grow only slowly in the laboratory so that it may take days or weeks to obtain cultures for identification. Some pathogens have yet to be grown at all in the laboratory so that their identification requires alternate methodologies.

The specific tests that are employed depend upon the target organism. Usually the specific suite of tests are selected based upon the suspected organism, which typically is based upon knowledge of disease symptoms in a patient or background epidemiological data. In the case of biological weapons detection, analyses are likely to be targeted at a relatively small list of pathogens known to have been considered for use in biological weapons development programs, symptoms of individuals in cases of unusual disease outbreaks, and/or specific intelligence information. While culture techniques are a standard epidemiological procedure that are likely to be used in the investigation of any unusual disease outbreaks, it must be noted that it has the greatest probability for loss of valuable proprietary strains of microorganisms. In a fermentation process, including the

production of pharmaceuticals worth millions of dollars, a proprietary microbial strain essentially represents an entire chemical factory, including the instructions for making the desired industrial product. Loss of valuable microbial strains could be very costly to industry and fear of such losses is responsible in large part for industrial resistance to the inclusion of on-site inspections as part of a verification regime for the Biological Weapons Convention. Eliminating such objections are likely to require safeguards that lessen the threat of loss of proprietary organisms. This may require use of analytical approaches that do not employ viable cultures.

Gene probes and amplification procedures can be used on site with nonviable microorganisms. This is a very powerful technique. However, it should be recognized that it is more difficult to protect the proprietary nature of the genetic information of a microorganism from loss during an inspection that uses such techniques. Enzymes are available that will degrade nucleic acid molecules; such endonuclease could be used to scramble the information, but if the nucleic acids are degraded too far they no longer are useful for diagnostic purposes. Compared to culture methods, the use of the polymerase chain reaction (PCR) and gene probes is about a hundred times more expensive than culture-based identification methods. It is more specific and faster than culture methods. Hence it is possible to rapidly screen for specific microorganisms on a list of potential biological agents. The specificity of molecular detection is advantageous in epidemiological investigations where recognition of a molecular signature can be linked to a source. This can be very powerful in investigations of alleged development or use of biological weapons. However, the high degree of specificity also means that there is increased likelihood of failing to detect a biological weapon; the method can be too specific. Even very minor changes in the nucleotide sequence of DNA can cause a failure in PCR amplification and gene probe detection.

The fact that PCR-gene probe analyses can be performed on nonviable microorganisms greatly reduces the risks of losing valuable industrial microorganisms. Samples can be boiled to kill the microorganisms before they are submitted for analysis. Although the use of molecular analyses of nonviable microorganisms offers increased protection against industrial loss of proprietary

cultures, there is still a danger of losing information about genetic sequences that may have industrial value. Hence there may still be industrial objections to this approach. Treatment with enzymes to digest DNA prior to sampling and analysis may alleviate these objections as they would minimize the risk of losing proprietary information. Specific endonuclease are available that could be used to scramble the genetic information, but if the nucleic acids are degraded too far they no longer are useful for diagnostic purposes. Digestion of DNA prior to analysis could mask the presence of some DNA sequences and hence could lower the effectiveness of molecular analyses aimed at revealing the presence of biological weapons.

Immunological methods can be used with appropriately prepared specimens to identify nonviable microorganisms. Killed microorganisms can still be identified by immunological testing to achieve compliance with the BWC and the detection of biological weapon agents. Immunological identification of potential biological weapon agents can confirm the presence or absence of suspected organisms with a high degree of confidence. Immunological identification of potential biological weapon agents can confirm the presence of absence of suspected organisms with a high degree of confidence. As with molecular approaches, serological methods that employ immune reactions can be used with appropriately prepared specimens to identify nonviable microorganisms. Killed microorganisms can still be identified by serological testing to achieve compliance with the BWC and the detection of biological weapon agents. DNA can be totally digested without losing identification capability, thereby eliminating the risk to industry of losing proprietary microorganisms or their genetic information. Thus, immunological identification could provide the balance needed for protection of proprietary cultures and the need to identify biological weapons.

Dr. Mahy should expand the serology section giving strengths and weaknesses.
Dr. Donlon should add a section on chemical detection.